Symposium paper / Article de symposium

Diverse traits for pathogen fitness in *Gibberella zeae*[1,2]

A.E. Desjardins and R.D. Plattner

Abstract: *Gibberella zeae* is an important pathogen of wheat, maize, and other cereal crops worldwide. Pathogen fitness in *G. zeae* is the outcome of selection for traits that increase its ability to survive and reproduce in plant pathosystems. Current research on mechanisms of pathogen fitness uses tools such as production of specific mutations by targeted gene disruption and analysis of genetic variation in natural populations. Gene disruption experiments indicate that production of the trichothecene deoxynivalenol (DON) enhances virulence on wheat and maize, and that production of sexual spores enhances head blight on wheat under field conditions. Natural populations from the U.S.A. and from Nepal differ significantly in virulence on wheat, sexual fertility, and trichothecene chemotype. Strains from both populations can produce DON, but only strains from Nepal can also produce nivalenol, which differs from DON by the addition of a hydroxyl group. Genetic analyses are underway to investigate associations of pathogen fitness of *G. zeae* with strain genotype, trichothecene chemotype, and other traits.

Key words: Fusarium graminearum, wheat, head blight, trichothecenes, mating-type genes, population genetics.

Résumé : Le *Gibberella zeae* est responsable d'importantes maladies du blé, du maïs et d'autres céréales dans le monde entier. L'adaptation du *G. zeae* à causer des maladies est le résultat de la sélection pour des caractères qui augmentent son aptitude à survivre et à se reproduire dans un pathosystème végétal. Les recherches actuelles sur les mécanismes d'adaptation à causer des maladies utilisent des outils tels que la production de mutations spécifiques par disruption génique avec des gènes cibles et l'analyse de la variation génétique dans les populations naturelles. Les expériences sur la disruption génique indiquent que la production de la trichothécène déoxynivalénol (DON) augmente la virulence sur le blé et le maïs et que la production de spores sexuelles accroît la brûlure des épis du blé en conditions naturelles. Des populations en provenance des États-Unis et du Népal diffèrent significativement sur le plan de la virulence envers le blé, de la fertilité sexuelle et du chimiotype de trichothécènes. Les souches des deux populations peuvent produire du DON, mais seules les souches du Népal peuvent aussi produire du nivalénol, qui diffère du DON par un groupe hydroxyle supplémentaire. Des analyses génétiques sont en cours pour rechercher des associations entre l'adaptation à causer des maladies chez le *G. zeae* et le génotype des souches, le chimiotype de trichothécènes et d'autres caractères.

Mots clés : Fusarium graminearum, brûlure des épis, blé, trichothécènes, gènes du type sexuel, génétique des populations.

Introduction

Gibberella zeae (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe) causes head blight of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and other small grains. This pathogen also infects maize (*Zea mays* L.) ears and stalks, and a diversity of other plants and plant tissues worldwide. *Gibberella zeae* reduces yields and also contaminates grain with trichothecene toxins that can be harmful to animals that consume infected grain. The pathogen was first described on wheat in North America in the late nineteenth century (Sutton 1982). More than a hun-

Accepted 22 December 2002.

A.E. Desjardins[3] and R.D. Plattner. Agricultural Research Service, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, 1815 North University Street, Peoria, IL 61604, U.S.A.

[1]This paper was a contribution to the symposium entitled Phytopathological Challenges, held during The Canadian Phytopathological Society Annual Meeting at Waterton Lakes National Park, Alta., June 2002.

[2]Disclaimer: Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the products, and the use of the name by U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

[3]Corresponding author (e-mail: desjarae@ncaur.usda.gov).

dred years later, *G. zeae* still causes outbreaks of wheat and barley head blight across the U.S.A. and Canada, and serious epidemics in the prairie states and provinces (Clear and Patrick 2002; Nganje et al. 2001). Research on controlling *G. zeae* diseases of wheat, barley, and maize has focused on developing host lines with improved resistance. Breeding for resistance has been hindered by the polygenic inheritance of resistance to *G. zeae* and by the impact of environment on disease. Identification of pathogen fitness traits of *G. zeae*, i.e., traits important for survival and pathogenicity, should assist in identification of host resistance mechanisms and environmental factors that affect levels of disease.

Current research on mechanisms of pathogen fitness of *G. zeae* relies on tools such as mutational and sexual recombinational analyses. *Gibberella zeae* is amenable to chemical mutagenesis (Leslie 1983) and to transformation-mediated gene disruption using methods and selectable markers that are standard for ascomycetes (Proctor et al. 1995). *Gibberella zeae* is homothallic, i.e., self-fertile, and can produce abundant perithecia with ascospores in the laboratory and in the field. Although *G. zeae* is homothallic, strains can be outcrossed under laboratory conditions to produce heterozygous perithecia and thus allow meiotic analysis (Bowden and Leslie 1999). *Gibberella zeae* is amenable to both random ascospore and tetrad analysis, and interpretation of segregation ratios is simplified by the fact that *G. zeae* is haploid. Although rather laborious, because strain markers are needed to identify heterozygous perithecia, sexual recombination in *G. zeae* has the potential to allow the characterization of a wide range of mutants and natural variants.

Recent DNA-marker analyses of natural populations from North and South America, Europe, and Asia indicate the presence of genetically divergent populations or lineages within *G. zeae* (Carter et al. 2000; O'Donnell et al. 2000; Gale et al. 2002; Zeller et al. 2001). Under laboratory conditions, however, strains from geographically widely separated populations can be interfertile and produce heterozygous perithecia with viable, recombinant ascospores (Bowden and Leslie 1999; Jurgenson et al. 2002). Thus, *G. zeae* may constitute a biological species as defined by Ernst Mayr (1970): "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups". On the other hand, *G. zeae* populations that retain the ability to interbreed under laboratory conditions may be genetically isolated in natural environments. Studies are underway to apply both biological and phylogenetic species concepts to natural populations of *G. zeae*. To assess the potential impact of either accidentally or deliberately introducing novel populations of *G. zeae* into agricultural systems in the U.S.A. and Canada, it will be important to determine also whether specific population genotypes are associated with traits important for pathogen fitness.

In this paper, we will present recent and current research based on specific gene disruption and analysis of natural genetic variation to investigate diverse traits for pathogen fitness in *G. zeae*. First, we will summarize studies that have used targeted gene disruption and sexual recombinational analyses to determine the importance of trichothecenes in virulence on wheat and maize. Second, we will present preliminary data from a study that is using targeted gene disruption to determine the importance of ascospores head-blight epidemics on wheat. Lastly, we will present data from population genetic analyses that compare *G. zeae* from the U.S.A. and from Nepal for genotype, trichothecene chemotype, sexual fertility, and virulence on wheat.

Trichothecene chemotypes and distribution

Trichothecenes are a large family of sesquiterpene epoxides that inhibit eukaryotic protein synthesis. They are produced by a number of *Fusarium* spp., and related fungi, and differ primarily in the extent and sites of oxygenation and degree of esterification. Two major trichothecene chemotypes of *G. zeae* have been identified (Table 1): producers of deoxynivalenol (DON) and producers of nivalenol (NIV), which has an additional hydroxyl group at C-4 (Fig. 1) (Marasas et al. 1984). Strains of *G. zeae* in the U.S.A., as well as in Canada, are of the DON chemotype (see Table 2), producing DON as the major trichothecene in laboratory cultures and in seeds from naturally or experimentally infected wheat (Table 1). 15-Acetyldeoxynivalenol is usually observed as a minor cometabolite; occasionally, small amounts of 3-acetyldeoxynivalenol are observed (Table 1). Strains of *G. zeae* found in Asia, Africa, and Europe can be of either the DON or NIV chemotype. In laboratory cultures, strains of the NIV chemotype produce principally NIV or 4-acetylnivalenol (4-acetylNIV or fusarenone-X) as the major trichothecene. In seeds from infected wheat, 4-acetylNIV is not observed (Table 1). Deoxynivalenol can also be produced by strains of the NIV chemotype, but it is usually present at less than 1% of the level of NIV or 4-acetylNIV.

Genes and pathway of trichothecene biosynthesis

The biosynthesis of trichothecenes by *Fusarium* spp. proceeds from farnesyl pyrophosphate via trichodiene through a complex series of steps to trichothecenes such as DON, NIV, and T-2 toxin. In common with biosynthetic genes for other fungal secondary metabolites, a number of trichothecene-pathway genes are closely linked and constitute a gene cluster. To date, 12 trichothecene biosynthetic genes, designated *TRI3* through *TRI14*, have been localized to a 30-kb region in *G. zeae* and *Fusarium sporotrichioides* (Sherb.) (Brown et al. 2002; Lee et al. 2002). One unlinked gene, *TRI101*, encodes an isotrichodermol 3-*O*-acetyltransferase that is required for biosynthesis of the more toxic trichothecene intermediates (Kimura et al. 1998; McCormick et al. 1999).

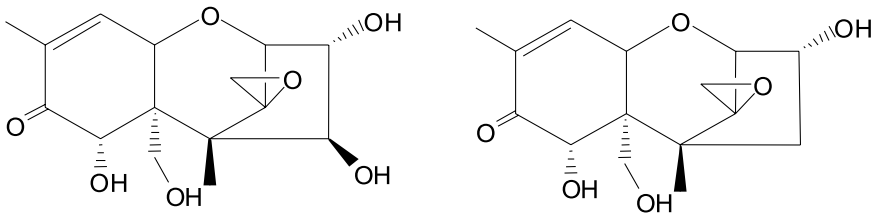
Sequence analysis indicates that the biosynthetic gene clusters of DON producers (from the U.S.A. or Asia) and NIV producers (from Asia) contain the same genes. However, the *TRI7* and *TRI13* homologues are nonfunctional in DON-producing strains (Brown et al. 2002; Lee et al. 2002). *TRI7* encodes a 4-acetyltransferase and *TRI13* encodes a 4-hydroxylase, which is consistent with the fact that strains of the NIV chemotype produce NIV and 4-acetylNIV, which are oxygenated at C-4. In contrast, strains of the DON chemotype have nonfunctional *TRI7* and *TRI13* genes and thus do not produce NIV, but rather produce

Table 1. Trichothecene chemotypes of *Gibberella zeae* from the U.S.A. and Nepal.

		Trichothecenes produced*		
Chemotype	Growth medium	Major	Minor (<10%)	Rare
Deoxynivalenol (DON)	Grain substrate	DON	15-AcetylDON	3-AcetylDON
	Infected wheat heads	DON	15-AcetylDON	3-AcetylDON
Nivalenol (NIV)	Grain substrate	NIV, 4-AcetylNIV	NIV, 4-AcetylNIV	DON
	Infected wheat heads	NIV		DON

*The ability of strains to produce trichothecenes on grain substrate was assessed, in cultures grown for 2–4 weeks on autoclaved rice, by gas chromatography – mass spectrometry (GC–MS) of trimethylsilyl derivatives as described by Carter et al. (2002) and Jurgenson et al. (2002). Compounds were identified by comparing their retention time and mass spectrum with authentic standards. The ability of strains to produce trichothecenes in infected wheat heads was assessed in samples of wheat seeds harvested from greenhouse virulence tests conducted as described by Desjardins et al. (2000a). Seeds from 10 replicate heads of each treatment were pooled, ground, and analyzed by GC–MS as described above.

Fig. 1. Structures of nivalenol and deoxynivalenol.



Nivalenol Deoxynivalenol

DON, which is not oxygenated at C-4. The genetic evidence supports the hypothesis that NIV production is ancestral to DON production in *G. zeae*.

Trichothecene-deficient mutants and virulence of *G. zeae* on wheat and maize

The acute phytotoxicity of DON, NIV, and other trichothecenes and their occurrence in plant tissues suggested the hypothesis that trichothecenes play a role in the plant pathogenesis of *G. zeae* (Eudes et al. 2000; Wang and Miller 1988). To test this hypothesis, trichothecene production was blocked in *G. zeae* by transformation-mediated disruption of *TRI5*, the gene for trichodiene synthase. Disruption of *TRI5* results in the production of mutants that are no longer able to produce DON or any trichothecene biosynthetic intermediates (Proctor et al. 1995). *TRI5* was disrupted by standard protoplast transformation with the hygromycin-resistant gene, *HygB*, as a selectable marker, generating two types of trichothecene-nonproducing mutants. In some mutants the *TRI5* gene was replaced with plasmid DNA by a double crossover event, while in others, the *TRI5* gene was disrupted by a single crossover event.

The virulence of both types of trichothecene-nonproducing mutants was compared with that of the trichothecene-producing progenitor strain by injection of macroconidia into wheat heads and maize ears. Both types of trichothecene-nonproducing mutants were substantially reduced in their ability to cause head blight on a wide range of wheat cultivars in greenhouse tests (Eudes et al. 2001; Proctor et al. 1995). Furthermore, trichothecene-nonproducing mutants were also reduced in their ability to cause head blight on wheat in field tests at two locations in the U.S.A. (Desjardins et al. 1996, 2000a). In addition, in field tests in the U.S.A. and Canada, trichothecene-nonproducing mutants were pathogenic on maize ears but appeared less able than the progenitor strain to spread within the ears (Harris et al. 1999).

Further genetic analyses have confirmed that the reduced virulence of *TRI5*-disruption mutants of *G. zeae* is caused by their inability to produce trichothecenes. A functional *TRI5* and trichothecene production were restored by *TRI5*-gene reversion in the gene-disrupted mutant and by *TRI5* complementation in the gene-deletion mutant. In both cases, high virulence on wheat and maize was also restored (Desjardins et al. 1996, 2000a; Harris et al. 1999). In addition, in a series of genetic crosses between trichothecene-nonproducing mutants and the progenitor strain, all progeny that had the disrupted *TRI5* allele did not produce DON and exhibited low virulence on wheat heads, whereas progeny with the wild-type *TRI5* allele produced DON and exhibited high virulence (Desjardins et al. 2000a).

Host resistance to trichothecenes

In principle, if production of trichothecenes increases pathogen virulence, then increased host resistance to trichothecenes should increase host resistance to the pathogen. Thus, plant genetic analysis should confirm the identification of trichothecenes as probable virulence factors. Genes that increase plant resistance to trichothecenes have been identified, and whether such genes can also increase plant resistance to *G. zeae* is under investigation. Two trichothecene-resistant genes are fungal genes that encode proteins reducing the toxicity of trichothecenes: *TRI101*, the gene encoding isotrichodermol 3-*O*-acetyltransferase, and *PDR5*, a yeast gene that is similar in function to *TRI12*, which encodes a multidrug-resistance transporter protein that is required for trichothecene biosynthesis (Alexander et

al. 1999). Transgenic expression of either *TRI101* or *PDR5* increased resistance of tobacco to trichothecenes (Muhitch et al. 2000). Wheat lines expressing *TRI101* have shown significantly increased resistance to head blight in greenhouse tests (Hohn et al. 2002; Okubara et al. 2002).

Trichothecenes are potent inhibitors of protein synthesis. Their mechanism of inhibition is believed to involve binding to the 60S ribosomal protein L3 (RPL3). In an effort to increase resistance to trichothecenes, a rice gene encoding RPL3 was modified to change amino acid 258 from tryptophan to cysteine, a change that confers resistance to trichothecenes to yeast. Transgenic expression of the modified *Rpl3* increased resistance of tobacco to trichothecenes (Harris and Gleddie 2001). Maize, wheat, and barley lines expressing the modified *Rpl3* gene are being constructed and tested for resistance to *G. zeae* (Harris and Gleddie 2001).

Sexual spores in head-blight epidemics on wheat

Epidemics of fusarium head blight in the U.S.A. and Canada have been associated with colonization of residues of host crops, especially maize stalks, with *G. zeae* (Sutton 1982). On colonized crop residues, *G. zeae* can produce two types of infective spores: sexual spores, which are called ascospores, and asexual spores, which are called macroconidia. By the early twentieth century, wind-dispersed ascospores and rain-dispersed macroconidia both were proposed to play important roles in infecting wheat heads in the field. To date, the relative importance of these two spore types in head-blight epidemics on wheat under different environmental conditions is not well understood. To investigate the importance of ascospores in head blight on wheat, we used targeted gene disruption to create mutants that cannot produce ascospores.

Sexual development and production of sexual spores in *G. zeae*, as in other ascomycetes, are controlled by the mating type (*MAT*) locus (Turgeon 1998). The homothallic *G. zeae MAT* locus carries both *MAT1-1* and *MAT1-2* genes localized to an 8.5-kb region. In *G. zeae*, the *MAT* locus has three *MAT1-1* genes, designated *MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3* and one *MAT1-2* gene, designated *MAT1-2-1* (Yun et al. 2000). DNA sequence analysis indicates that *MAT* genes encode transcriptional regulators of sexual development. Deletion of the entire *MAT* locus of *G. zeae* has created mutants that cannot produce ascospores, but appear similar to the progenitor strain in colony morphology and in production of macroconidia and trichothecenes. In greenhouse tests, macroconidia of *MAT*-deletion, ascospore-nonproducing mutants were able to cause blight after injection into wheat heads (Desjardins et al. 2001).

In field tests in Illinois in 2001 and 2002, ascospore-nonproducing mutants were compared with the ascospore-producing, progenitor strain of *G. zeae* for ability to cause head-blight epidemics on wheat (Desjardins et al. 2001). To provide inoculum, autoclaved maize stalk pieces were inoculated with fungal strains and incubated in the laboratory. After 1 month, the progenitor strain produced abundant perithecia on stalk pieces, while the *MAT*-deletion mutants produced none. In field tests in 2001 and 2002, infected stalk pieces were placed on the ground in plots of susceptible spring-wheat cultivars 'Wheaton' (in 2001) and 'Norm' (in 2002), approximately 3 weeks before flowering. Results from 2001 indicate that the *MAT*-deletion mutant was significantly less effective than the progenitor strain in infecting wheat heads, in reducing yield, and in increasing DON levels of harvested seeds. Analyses of wheat heads from the 2002 field test are in progress. For the 2003 season, additional field tests of *G. zeae MAT*-deletion and *MAT*-complementation mutants are planned to further assess the importance of ascospores in head-blight epidemics on wheat.

Comparison of *G. zeae* from the U.S.A. and Nepal

Biological diversity

Morphological, biological, and phylogenetic species concepts all identify *G. zeae* isolated from wheat and maize across the U.S.A. as one polymorphic but interbreeding population, which has been designated lineage 7 (Bowden and Leslie 1999; Carter et al. 2002; O'Donnell et al. 2000; Zeller et al. 2001). Numerous analyses of *G. zeae* from the U.S.A. and Canada indicate that this population is dominated by strains that produce high levels of DON and large numbers of ascospores and are highly virulent on wheat and maize (Marasas et al. 1984). Thus, this North American population appears to show selection for limited diversity in expression of three traits that have been associated with pathogen fitness: DON production, sexual fertility, and virulence.

The biological diversity of *G. zeae* populations from outside North America and from sources other than infected wheat and maize is less well characterized. To study fungal diversity, we are examining *G. zeae* strains from a range of plant species collected largely at one site (<12 km^2) in Lamjung district, in the foothills of the Himalayas, in Nepal. This environment provides great variation in altitude, rainfall, and geology, and supports a complex agroecosystem of subsistence farms with largely local landraces of wheat (*Triticum* spp.), maize, rice (*Oryzae* spp.), and other grains. *Gibberella zeae* infects a wide range of plant species in the sampling area and 600 strains have been isolated from samples of wheat, maize, and rice seed, as well as from crop debris and weeds (Desjardins et al. 2000b, 2000c; A.E. Desjardins and R.D. Plattner, unpublished data). Results to date indicate that the *G. zeae* population from Nepal displays unusually high genetic diversity as well as extensive diversity in trichothecene chemotype, sexual fertility, and virulence on wheat.

A collection of 40 strains of *G. zeae* from wheat seed in Illinois was selected to represent the U.S.A. population to directly compare *G. zeae* from two very different environments. For the purposes of this comparison, the Illinois strains are designated as U.S.A. strains and the Lamjung district strains are designated as Nepal strains.

Table 2. Frequency distribution of head-blight severity on wheat for *Gibberella zeae* from the U.S.A. and Nepal.

			Percentage of strains with each blight rating†				
Source*	Chemotype†	Number of strains	0–20	21–40	41–60	61–80	81–100
U.S.A.	Deoxynivalenol	40	2	0	0	2	96
Nepal	Deoxynivalenol	76	4	8	11	18	59
Nepal	Nivalenol	178	18	18	20	22	22

*Strains were isolated from four wheat seed samples collected in Illinois in 1996; from numerous wheat, maize, and rice seed samples collected in Nepal in 1993, 1997, and 2000; and from soil debris and weed samples collected in Nepal in 2000 (Desjardins et al. 2000b, 2000c; A.E. Desjardins and R.D. Plattner, unpublished data).

†Strains were scored for virulence on wheat heads in greenhouse tests as previously described (Desjardins et al. 2000a). Data are expressed as mean percentage of blighted spikelets per head 17–18 days after inoculation. For trichothecene analysis, seeds from 10 replicate heads of each treatment were pooled, ground, and analyzed by GC–MS as described in footnote to Table 1.

Trichothecene chemotypes

A first objective was to compare trichothecene chemotypes of strains from the U.S.A. and Nepal (Table 2). As expected, all 40 U.S.A. strains were of the DON chemotype. In laboratory cultures and in seeds from infected wheat heads, all strains produced DON. No NIV was detected in cultures or in wheat seeds infected by any of the strains from Illinois. Two hundred fifty-four of the 261 Nepal strains examined produced trichothecenes in laboratory culture and (or) in seeds from infected wheat heads: 30% produced primarily DON and 70% produced primarily NIV. Nivalenol-producing strains comprised from 67 to 74% of strains isolated from each of the five sample types (wheat, maize, rice, soil debris, and weeds). The cooccurrence of strains with DON and NIV chemotypes in Nepal is consistent with previous reports of both chemotypes of *G. zeae* in China, Japan, and Korea (Lee et al. 2002; Marasas et al. 1984; O'Donnell et al. 2000).

Sexual fertility of strains

A second objective was to compare sexual fertility of strains of *G. zeae* from the U.S.A. and Nepal. The Nepal strains were selected to represent both trichothecene chemotypes and each of the five sample types (wheat, maize, rice, soil debris, and weeds). Sexual fertility was assessed by growing strains for 2 months on carrot agar plates, with two plates per strain (Bowden and Leslie 1999). The 40 DON-producing U.S.A. strains were highly self-fertile with 98% producing abundant perithecia with mature ascospores within 1 month. In contrast, 51% of 47 DON-producing strains and 60% of 74 NIV-producing strains examined from Nepal produced no perithecia with mature ascospores, even after 2 months. The frequency of nonfertile strains varied among sample types, from 40 to 50% for wheat, maize, and rice, to 64% for crop debris, and 79% for weeds.

Virulence of macroconidia injection on wheat

A third objective was to compare strains of *G. zeae* from the U.S.A. and Nepal for their ability to cause head blight on wheat following injection of macroconidia (Table 2). Ninety-six percent of the 40 U.S.A. strains were highly virulent, causing a rating of more than 80% spikelets blighted. Sixty percent of the 76 DON-producing Nepal strains were highly virulent, but this group of strains was, on average, less virulent than DON-producing U.S.A. strains ($p < 0.01$). In contrast, only 21% of the 178 NIV-producing Nepal strains were highly virulent, and this group of strains was significantly less virulent than DON-producing strains from the U.S.A. or Nepal ($p < 0.01$).

Associations among trichothecene chemotype, strain genotype, and virulence

The survey of trichothecene chemotype and virulence among 254 *G. zeae* strains from Nepal identified a strong association of high virulence with the DON chemotype. This association, however, may be fortuitous; recent DNA marker analyses indicate that two or more genetically divergent populations are present in this collection of *G. zeae* from Nepal. In one analysis, 62 of the strains from wheat, maize, and rice were placed into two genetically distinct groups, designated A and B (Carter et al. 2000). Groups A and B were then shown to be genetically distinct from group C, which contains strains from the U.S.A. and Europe (Carter et al. 2002). In an independent analysis, four of the strains from maize were placed into three different lineages, designated lineages 2, 3, and 6, all of which are distinct from U.S.A. lineage 7 (O'Donnell et al. 2000).

Two collaborative projects have been undertaken to analyze associations between strain genotype, trichothecene chemotype, and virulence of *G zeae* from the U.S.A. and Nepal. In the first study, 28 U.S.A. and 36 Nepal strains were compared for trichothecene chemotype and virulence, and for Group A, B, or C genotype (Table 3). As expected, strains from group C produced only DON and were highly virulent. Strains from group A were equally likely to produce DON or NIV and group A strains of both chemotypes were less virulent than group C strains in wheat and maize seedling tests (Carter et al. 2002) and in head-blight tests on wheat (Table 3). Strains from group B all produced NIV and were less virulent than group A strains of both chemotypes (Carter et al. 2002 and Table 3). Thus, in this initial study of biological diversity of *G. zeae* from Nepal, virulence on wheat heads was associated with strain genotype and trichothecene chemotype. A more extensive study of biological diversity of *G. zeae* from Nepal is underway in collaboration with Andrew Jarosz of Michigan State University, East Lansing, Mich. Our objectives are to analyze the collection of 600 strains for trichothecene chemotype and for virulence in head-blight tests on wheat and to determine the fine genetic structure of the population, using amplified fragment-length polymorphisms. This work should allow us to determine whether Nepal is a center of diversity for *G. zeae* and to evaluate the potential for generation of

Table 3. Relationships between genotype, trichothecene chemotype, and head-blight severity on wheat for *Gibberella zeae* from the U.S.A. and Nepal.

Source*	Chemotype†	Number of strains	Genotype group‡	Percentage spikelet blight†
U.S.A.	Deoxynivalenol	28	C	94±13 a
Nepal	Deoxynivalenol	15	A	60±30 b
Nepal	Nivalenol	11	A	44±30 b
Nepal	Nivalenol	10	B	10±6 c

*As described in Table 2.

†Strains were scored for virulence and trichothecene chemotype as described in footnotes to Tables 1 and 2. Means ± SD followed by the same letter are not significantly different at $p < 0.01$, according to the analysis of variance followed by comparison of groups, using least squares means.

‡Genotype data from Carter et al. (2000, 2002). Genotypes were determined by RAPD markers and by a sequence-characterized amplified region polymorphism.

novel genotypes of concern for head-blight management on wheat worldwide.

Conclusion

"Every local population is adapted, through natural selection, to the specific environment in which it lives" (Mayr 1970). With their large population sizes and short generation times, fungal species such as *G. zeae* are amenable to extensive natural variation by mutation and sexual recombination. Comparison of two natural populations of *G. zeae*, one isolated from wheat in the U.S.A. and the other from wheat and other plant hosts in Nepal, shows that there are large differences in biological diversity. The two populations differ in genetic structure, virulence on wheat, and traits such as sexual fertility and trichothecene production, all of which, as gene disruption experiments have indicated, may play important roles in pathogen fitness.

Certain traits such as high virulence and high sexual fertility confer obvious selective advantages to a plant pathogen. In addition, analysis of strains of *G. zeae* from Nepal indicates that higher virulence on wheat is associated with the DON chemotype. Comparison of trichothecene gene clusters indicates that gene organization is highly conserved for *F. sporotrichioides* and *G. zeae*, except that genes involved in C-4 oxygenation and acetylation are inactivated in DON-producing strains of *G. zeae* from the U.S.A., Nepal, and Korea (Brown et al. 2002; Lee et al. 2002). These data support the hypotheses that NIV production is the ancestral trait and that DON producers were derived from NIV producers. However, the distribution of DON-producing strains worldwide suggests that DON production is of considerable phylogenetic antiquity and may have some selective advantage. Efforts are underway to use *TRI* gene disruption and sexual recombinational analysis to study associations between trichothecene chemotypes and virulence of *G. zeae*.

Biological diversity of *G. zeae* provides material invaluable for investigating traits for pathogen fitness. But remarkable biological diversity is interesting in its own right. Explanations for the unusually high level of genetic and phenotypic variation of *G. zeae* from Nepal involve many factors, but few are amenable to rigorous analysis. If the diverse populations are native to Nepal, then selection factors in the shared environment that allow populations to occupy different ecological niches or otherwise avoid direct competition are unknown. If one or more of the populations are recent introductions via a host plant, then a comparison of intraspecific genetic variation might identify fewer polymorphisms in a *G. zeae* population on a more recently introduced host, such as maize, than on a native host, such as wheat or rice. This approach, however, is complicated by the ability of *G. zeae* strains to infect multiple hosts, and by multiple introductions of maize into Nepal dating from the early seventeenth century.

Although *G. zeae* is already widespread in the U.S.A. and Canada, the results from this and other studies indicate that the potential impact of accidental or deliberate introduction of strains from other *G. zeae* populations should be assessed. Information is needed especially for virulence, sexual fertility, trichothecene chemotype, and other traits that could affect the survival of introduced *G. zeae* and its impact on agricultural systems in North America.

References

Alexander, N.J., Hohn, T.M., and McCormick, S.P. 1999. *TRI12*, a trichothecene efflux pump from *Fusarium sporotrichioides*: gene isolation and expression in yeast. Mol. Gen. Genet. 261: 977–984.

Bowden, R.L., and Leslie, J.F. 1999. Sexual recombination in *Gibberella zeae*. Phytopathology, 89: 182–188.

Brown, D.W., McCormick, S.P., Alexander, N.J., Proctor, R.H., and Desjardins, A.E. 2002. Inactivation of a cytochrome P-450 is a determinant of trichothecene diversity in *Fusarium* species. Fungal Genet. Biol. 36: 224–233.

Carter, J.P., Rezanoor, H.N., Desjardins, A.E., and Nicholson, P. 2000. Variation in *Fusarium graminearum* isolates from Nepal associated with their host of origin. Plant Pathol. (London), 49: 452–460.

Carter, J.P., Rezanoor, H.N., Holden, D., Desjardins, A.E., Plattner, R.D., and Nicholson, P. 2002. Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. Eur. J. Plant Pathol. 108: 573–583.

Clear, R., and Patrick, S. 2002. Fusarium head blight in Canada [online]. Mycology, Canadian Grain Commission, Winnipeg, Man. Available from http://www.grainscanada.gc.ca/pubs/fusarium/fusarium-e2.htm [updated 7 Feb. 2003; accessed 9 Feb. 2003].

Desjardins, A.E., Proctor, R.H., Bai, G., McCormick, S.P., Shaner, G., Buechley, G., and Hohn, T.M. 1996. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant–Microbe Interact. 9: 775–781.

Desjardins, A.E., Bai, G., Plattner, R.D., and Proctor, R.H. 2000a. Analysis of aberrant virulence of *Gibberella zeae* following transformation-mediated complementation of a trichothecene-

deficient (*Tri5*) mutant. Microbiology (Reading, U.K), 146: 2059–2068.

Desjardins, A.E., Manandhar, H.K., Plattner, R.D., Manandhar, G.G., Poling, S.M., and Maragos, C.M. 2000b. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. Appl. Environ. Microbiol. 66: 1020–1025.

Desjardins, A.E., Manandhar, G., Plattner, R.D., Maragos, C.M., Shrestha, K., and McCormick, S.P. 2000c. Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. J. Agric. Food Chem. 48: 1377–1383.

Desjardins, A.E., Brown, D.W., Yun, S.-H., Plattner, R.D., Lee, T., and Turgeon, G. 2001. Using a *Gibberella zeae Mat*-locus knockout to test the importance of ascospores in a wheat FHB field epidemic. *In* Canadian Workshop on Fusarium Head Blight. 3–5 Nov. 2001, Ottawa, Ont. Available from Linda Harris, Eastern Cereal and Oilseed Research Centre, Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa, Ont. K1A 0C6. (Abstr.)

Eudes, F., Comeau, A., Rioux, S., and Colin, J. 2000. Phytotoxicité de huit mycotoxines associées à la fusariose de l'épi chez le blé. Can. J. Plant Pathol. 22: 286–292.

Eudes, F., Comeau, A., Rioux, S., and Collin, J. 2001. Impact of trichothecenes on Fusarium head blight (*Fusarium graminearum*) development in spring wheat (*Triticum aestivum*). Can. J. Plant Pathol. 23: 318–322.

Gale, L.R., Chen, L.-F., Hernick, C.A., Takamura, K., and Kistler, H.C. 2002. Population analysis of *Fusarium graminearum* from wheat fields in Eastern China. Phytophathology, 92: 1315–1322.

Harris, L.J., and Gleddie, S.C. 2001. A modified *Rpl3* gene from rice confers tolerance of the *Fusarium graminearum* mycotoxin deoxynivalenol to transgenic tobacco. Physiol. Mol. Plant Pathol. 58: 1–9.

Harris, L.J., Desjardins, A.E., Plattner, R.D., Nicholson, P., Butler, G., Young, J.C., Weston, G., Proctor, R.H., and Hohn, T.M. 1999. Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Dis. 83: 954–960.

Hohn, T.M., Peters, C., and Salmeron, J. 2002. Trichothecene-resistant transgenic plants. U.S. Patent and Trademark Office (USPTO) full-text and image database [online]. USPTO, U.S. Department of Commerce, Washington, D.C., U.S. patent No. 6 346 655, 12 February 2002. Available from http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=/netahtml/srchnum.htm&r=1&f=G&l=50&s1=6,346,655.WKU.&OS=PN/6,346,655&RS=PN/6,346,655 [accessed 9 Feb. 2003].

Jurgenson, J.E., Bowden, R.L., Zeller, K.A., Leslie, J.F., Alexander, N.J., and Plattner, R.D. 2002. A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics, 160: 1451–1460.

Kimura, M.I., Kaneko, I., Komiyama, M., Takatsuki, A., Koshino, H., Yoneyama, K., and Yamaguchi, I. 1998. Trichothecene 3-*O*-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins. Cloning and characterization of *Tri101*. J. Biol. Chem. 273: 1654–1661.

Lee, T., Han, Y.-K., Kim, K.-H., Yun, S.-H., and Lee, Y.-W. 2002. *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Appl. Environ. Microbiol. 68: 2148–2154.

Leslie, J.F. 1983. Some genetic techniques for *Gibberella zeae*. Phytopathology, 73: 1005–1008.

Marasas, W.F.O., Nelson, P.E., and Toussoun, T.A. (*Editors*). 1984. Toxigenic *Fusarium* species: identity and mycotoxicology. The Pennsylvania State University Press, University Park, Pa.

Mayr, E. 1970. Populations, species, and evolution. Harvard University Press, Cambridge, Mass. pp. 12 and 94.

McCormick, S.P., Alexander, N.J., Trapp, S.E., and Hohn, T.M. 1999. Disruption of *TRI101*, the gene encoding trichothecene 3-*O*-acetyltransferase, from *Fusarium sporotrichioides*. Appl. Environ. Microbiol. 65: 5252–5256.

Muhitch, M.J., McCormick, S.P., Alexander, N.J., and Hohn, T.M. 2000. Transgenic expression of the *TRI101* or *PDR5* gene increases resistance of tobacco to the phytotoxic effects of the trichothecene 4,15-diacetoxyscirpenol. Plant Sci. (Shannon), 157: 201–207.

Nganje, W.E., Johnson, D.D., Wilson, W.W., Leistritz, F.L., Bangsund, D.A., and Tiapo, N.M. 2001. Economic impacts of Fusarium head blight in wheat and barley: 1998–2000 [online]. U.S. Wheat and Barley Scab Initiative, Michigan State University, East Lansing, Mich. Agribusiness and Applied Economic, Report No. 464, Department of Agribusiness and Applied Economic, Agriculture Experiment Station, North Dakota State University, Fargo, N.D. Available from http://www.scabusa.org/pdfs_dbupload/98-00_Economics_Report.PDF [accessed 9 Feb. 2003].

O'Donnell, K., Kistler, H.C., Tacke, B.K., and Casper, H.H. 2000. Gene genealogies reveal global phylogenetic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. U.S.A. 97: 7905–7910.

Okubara, P.A., Blechl, A.E., McCormick, S.P., Alexander, N.A., Dill-Macky, R., and Hohn, T.M. 2002. Expression of *FsTRI101*, a gene from *Fusarium sporotrichioides* encoding trichothecene acetyltransferase, in hexaploid wheat (*Triticum aestivum*). Theor. Appl. Genet. 106: 74–83.

Proctor, R.H., Hohn, T.M., and McCormick, S.P. 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Mol. Plant–Microbe Interact. 8: 593–601.

Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4: 195–209.

Turgeon, B.G. 1998. Application of mating type gene technology to problems in fungal biology. Annu. Rev. Phytopathol. 36: 115–137.

Wang, Y.Z., and Miller, J.D. 1988. Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathol. (Berlin), 122: 118–125.

Yun, S.-H., Arie, T., Kaneko, I., Yoder, O.C., and Turgeon, B.G. 2000. Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella/Fusarium* species. Fungal Genet. Biol. 31: 7–20.

Zeller, K.A., Vargas, J.I., Lee, Y.-W., Bowden, R.L., and Leslie, J.F. 2001. Comparison of populations of *Gibberella zeae* from Korea and North and South America. *In* 2001 National Fusarium Head Blight Forum Proceedings. 8–10 Dec. 2001, Erlanger, Ky. Organized by U.S. Wheat and Barley Scab Initiative, Michigan State University, East Lansing, Mich. Available from http://www.scabusa.org/pdfs/01_Proc_Complete_Web.pdf [accessed 9 Feb. 2003]. Kinko's, Okemos, Mich. p. 163 (Abstr.).